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Applications of Magnetic Nonolipase on the Synthesis of Vitamin A Palmitate and Hydrolysis of Olive Oil

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Abstract

Magnetic Nanolipase (MNL) was prepared previously in our lab and the potential applications were explored in the present work. Two types of reactions including hydrolysis of olive oil and synthesis of vitamin A palmitate were chosen to check the properties of MNL. When concentration of olive oil was 25% (v/v) immobilized lipase has the highest activity (80.7 U/g) under 50°C. The optimized conditions were as follows: Molecular ratio of palmitic acid and Vitamin A acetate was 2.5, reaction temperature and time were 35°C and 10 h, respectively. The highest yield of 51.4% was obtained for synthesis of vitamin A palmitate. All these results strongly suggest that MNL has potential industrial applications including production of pharmaceutical intermediates, cosmetics and food.

Introduction

Lipase (EC 3.1.1.3) is one of the most important enzymes and used as biocatalysts in the industry [1]. It catalyzes many reactions including hydrolysis of ester [2], aldol addition [3], ring-open polymerization [4], racemization [5], epoxidation [6]. However, free lipase can't be reused because the enzyme comes from fermentation technology [7]. In order to make it recyclable and lower the cost, immobilization is the best way among all the methods [8]. At present, the lipase has been immobilized on supports by physical adsorption [9] and covalent immobilization [10].

In our previous work [11], a simple method for immobilization of lipase on magnetic nanoparticles was developed. The immobilized Magnetic Nanopliase (MNL) has better thermal and pH stability than that of free lipase. Moreover, MNL can be recycled easily by external magnetic force. It is necessary to explore the potential applications of MNL because of the excellent characteristics.

In this work, MNL was employed as biocatalyst for hydrolysis of olive oil and synthesis of vitamin A palmitate. Novozym435, the most successful immobilized lipase, was used as the control to catalyze the same biocatalytic reactions.

Experimental

Chemicals

Olive oil, retinyl acetate, vitamin A palmitate and polyvinyl alcohol were purchased from SinoPharm Regent Co. Ltd (Shanghai, China). Novozym 435 was obtained from Novo Nordisk (Guangzhou, China). And p-Nitrophenyl palmitate was obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals were analytic or biological grade.

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Enzyme assay

Immobilized MNL was prepared according to the previous report with some modifications [11]. Typically, the aldehyde-functionalized Fe_3O_4@SiO_2 nanoparticles were suspended in 0.3 mL of the enzyme solution (0.39 mg mL^{-1}) and mixed well at 10°C, pH 6.0. After 24 h of incubation, the immobilized MNL was then filtered and washed 5 times with the pH 7.0 50 mmol L^{-1} PBS.

Lipase activity was determined as the previous report [12] with slight modification. Dissolve 0.5 g of p-Nitrophenyl palmitate (pNPP) in 100 mL of ethanol as the substrate to determine lipase activity. During the reaction, the release of p-Nitrophenol leads to the increase in absorbance at 410 nm which could be measured spectrophotometrically. About 20 mg of immobilized lipase was added to a mixture of 0.04 mL of 5 mmol L^{-1} pNPP solutions and 0.4 mL of 0.05 M phosphate buffer (PBS, pH 9.0) and incubated for 5 min at 45°C. The mixture was centrifuged for 10 min (10,000 rpm) to terminate the reaction. Diluted 0.5 mL of the supernatant 10-folds with distilled water, and measured at 410 nm in a spectrophotometer (UV-2450/2550, Shimadzu, Japan). One unit (U) of enzyme activity was defined as the amount of enzyme which catalyzed the production of 1 mmol p-Nitrophenol per minute under the experimental conditions.

Enzymatic synthesis of vitamin a palmitate

Enzymatic synthesis of vitamin A palmitate with prepared MNL was carried out in a 50 mL flask. Dissolved 2.5 mmol palmitic acid in 15 mL petroleum ether with sonicator bath, then poured into the flask. Added one mmol of retinyl acetate to the solutions, and kept stirring until retinyl acetate was dissolved. About 60 mg MNL was added to start the enzymatic reaction in a shaker. The temperature was maintained at 35°C when the reaction was performed. After the reaction, the samples were withdrawn and subjected for analysis with HPLC (SPD-20A, Shimadzu) equipped with a UV detector and an Agilent Microsorb-MV 100-5 C18 column. The injection volume was 20 µL and eluted with methanol at 1 mL min^{-1} at 30°C.

Enzymatic hydrolysis of olive oil

Olive oil and 4% polyvinyl alcohol was mixed and emulsified as the substrate for MNL hydrolysis. The emulsified olive oil was added into a 50 mL round bottom tube and kept the temperature at 50°C. Then MNL was added to start the reaction, the reaction was terminated by centrifugation 5 min at 4000 rpm. The supernatant was analyzed with phenolphthalein as the indicator for titration with 0.05 M NaOH. The reaction rate of enzymatic hydrolysis was calculated according to the volume of consumed NaOH.

Results and discussion

Lipase is known as a versatile biocatalyst which catalyzes many types of reactions at mild conditions [13]. In our previous work, lipase was immobilized onto modified magnetic nanoparticles successfully [11]. In the present work, two categories reactions including hydrolysis and synthesis were investigated to test the potential applications of MNL.

Hydrolysis of olive oil with MNL

In order to check the possibility for hydrolysis of olive oil, the factors like temperature and substrate concentration were investigated. As Figure 1 shown, the maximum reaction speed was obtained at 50°C. For comparison, Novozym435 was also tested and the maximum enzyme activity was observed at 40°C. The highest reaction speed of hydrolysis of olive oil with MNL was 25% higher than that of Novozym 435. The concentration of olive oil ranged from 5-30% was investigated to check the enzyme activity. Both enzymes had the maximum rate when the concentration of olive oil was 25%. MNL showed much higher reaction activity than that of Novozym435 as indicated in Figure 2. Novozym435 was used as the comparison because it is the most popular immobilized lipase developed by Degussa [14]. From these results, it is easy to conclude that MNL has better catalytic effect on the hydrolysis of olive oil. And it could be used for biodiesel production with olive oil as the substrate.

Synthesis of vitamin a palmitate with MNL

Vitamin A palmitate is one of the stable forms of Vitamin. In order to convert vitamin C to Vitamin A palmitate with MNL, the reaction conditions were optimized. Firstly, the ratio of palmitic acid to retinyl acetate was checked as shown in Figure 3. When the ratio of palmitic acid to retinyl acetate was 2.5, the highest yield of 51.4% was achieved. And the reaction time course was observed in Figure 4. The yield reached 51.4% when the reaction time was 10 hours. Effect of temperature on the reaction was shown in Figure 5, the yield was increased with temperature increasing from 25 to 35°C, and then decreased when the temperature was increased from 35 to 45°C. The highest yield of 51.4% was obtained at 35°C. Compared with the previous reports with Lipozyme RMIM, LA201056 and Lipozyme TLIM [15], the yield of vitamin A palmitate was much higher.

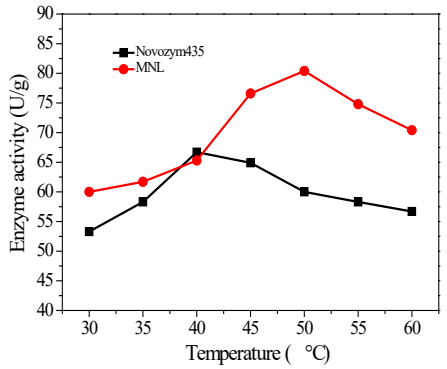

Figure 1: Effect of temperature on hydrolysis of olive oil.

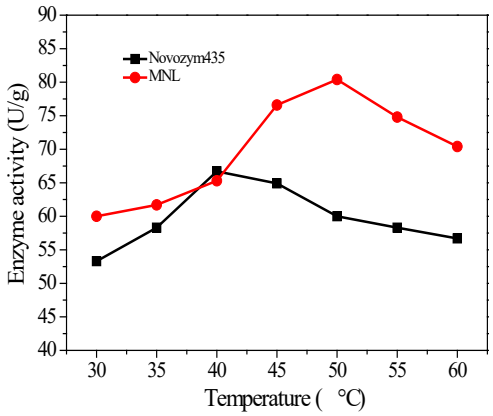

Figure 2: Effect of substrate concentration on the hydrolysis of olive oil.

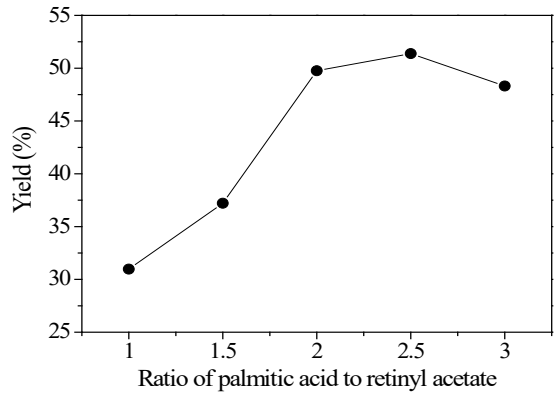

Figure 3: Effect of substrate molar ratio on the synthesis of vitamin A palmitate.

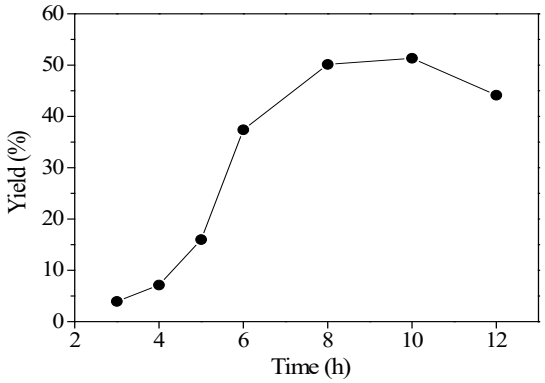

Figure 4: Effect of reaction time on the synthesis of vitamin A palmitate.

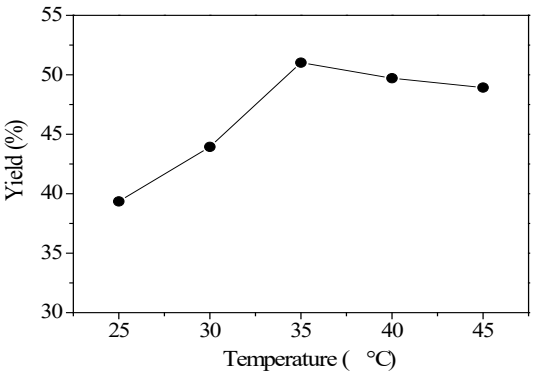

Figure 5: Effect of temperature on yield of vitamin A palmitate.

Conclusions

Magnetic Nanolipase (MNL) was used to explore the possible applications in the chemical, pharmaceutical and cosmetic industries. Enzymatic hydrolysis of olive oil and synthesis of viatamin A palmitate were chosen as the model reactions to test the characteristics of MNL. The results indicated that MNL were better than the commercial immobilized lipase (Novozym 435), and suggested that MNL has great potential applications in the industry.

References

1. Ávila SNS, Gutarra MLE, Fernandez-Lafuente R, Cavalcanti EDC, Freire DMG. Biochem. Eng. J. 2019; 144: 1-7.
2. Adlercreutz P. Chem. Soc. Rev. 2013; 42: 6406.
3. Branneby C, Carlqvist P, Hult K, Brinck T, Berglund P. J. Mol. Catal. B: Enzymatic. 2004; 31: 123.
4. Takwa M, Xiao Y, Simpson N, Malmström E, Hult K, et al. Biomacromolecules. 2008; 9: 704.
5. Ghanem A, Aboul-Enein HY. Chirality. 2005; 17: 1.
6. da Silva JMR, M. da G. Nascimento, Process Biochem. 2012.
7. Say R, Kecili R, Bicen O, Sisman FY, Hür D, et al. Process Biochem. 2011; 46: 1688.
8. Karimpil JJ, Melo JS, D'Souza SF. Int. J. Biol. Macromol. 2011.
9. Carvalho T, Adejanildo da S. Pereira, Renata C.F. Bonomo, Marcelo Franco, Priscilla V. Finotelli, Priscilla F. F. Amaral. Int. J. Biol. Macromol. 2020; 160: 889-902.
10. Weltz JS, Kienle DF, Schwartz DK, Kaar JL. ACS Catal. 2019; 9: 4992-5001.
11. Liu W, F. Zhou, X.-Y. Zhang, Y. Li, X.-Y. Wang, X.-M. Xu, et al. J. Nanosci. Nanotechnol. 2014; 14: 3068.
12. Chiou SH, Wu WT. Biomaterials. 2004; 25: 197.
13. Hasan F, Shah AA, Hameed A. Enzyme Microb. Technol. 2006; 39: 235.
14. Houde A, Kademi A, Leblanc D. Appl. Biochem. Biotechnol. 2004; 118: 155.
15. Liu T, Yin C, Tan T. Modern Chem. Ind. 2005; 25: 37.